Brazilian Journal of Development

Low power and short time under ultrasonic effect increase the inulinase activity

Baixa potência e pouco tempo sob efeito ultra-sônico aumentam a atividade da inulinase

Recebimento dos originais: 28/02/2019
Aceitação para publicação: 01/03/2019

Fábio Spitza Stefanski
Graduando em Engenharia Ambiental e Sanitária
Instituição: Universidade Federal da Fronteira Sul
Endereço: Laboratório de Microbiologia e Bioprocessos, ERS 135 - Km 72, 200, Caixa Postal 764,CEP 99700-970, Erechim, RS, Brasil
E-mail: fabio_stefanski@hotmail.com

Thamarys Scapini
Mestranda em Ciência e Tecnologia Ambiental
Instituição: Universidade Federal da Fronteira Sul
Endereço: Laboratório de Microbiologia e Bioprocessos,ERS 135 - Km 72, 200, Caixa Postal 764,CEP 99700-970, Erechim, RS, Brasil
E-mail: thami.scapini01@gmail.com

Aline Frumi Camargo
Mestranda em Ciência e Tecnologia Ambiental
Instituição: Universidade Federal da Fronteira Sul
Endereço: Laboratório de Microbiologia e Bioprocessos,ERS 135 - Km 72, 200, Caixa Postal 764,CEP 99700-970, Erechim, RS, Brasil
E-mail: alinefrumi@gmail.com

Jessica Zanivan
Graduando em Engenharia Ambiental e Sanitária
Instituição: Universidade Federal da Fronteira Sul
Endereço: Laboratório de Microbiologia e Bioprocessos,ERS 135 - Km 72, 200, Caixa Postal 764,CEP 99700-970, Erechim, RS, Brasil
E-mail: jessica.zanivan@hotmail.com

Karina Paula Preczeski
Mestranda em Ciência e Tecnologia Ambiental
Instituição: Universidade Federal da Fronteira Sul
Endereço: Laboratório de Microbiologia e Bioprocessos,ERS 135 - Km 72, 200, Caixa Postal 764,CEP 99700-970, Erechim, RS, Brasil
E-mail: karinapreczeski@hotmail.com

Gislaine Fongaro
Doutora em Biotecnologia pela Universidade Federal de Santa Catarina
Instituição: Universidade Federal de Santa Catarina (UFSC)

Brazilian Journal of Development

Endereço: Centro de Ciências Biológicas (CCB), Departamento de Microbiologia, Imunologia e Parasitologia (MIP), Laboratório de Virologia Aplicada, Florianópolis, SC, 88040-970 – Brasil

Helen Treichel
Doutora em Engenharia de Alimentos pela Universidade Estadual de Campinas
Instituição: Universidade Federal da Fronteira Sul
Endereço: Laboratório de Microbiologia e Bioprocessos, ERS 135 - Km 72, 200, Caixa Postal 764,CEP 99700-970, Erechim, RS, Brasil
E-mail: helentreichel@gmail.com

RESUMO

A produção de frutose e frutooligossacarídeos é de extrema importância industrial, uma vez que contemplam um seleto grupo de alimentos funcionais, sendo obtidos pela ação catalítica das inulinases. Os tratamentos ultrassônicos (TUS) têm sido amplamente utilizados em reações enzimáticas em váriasárea, com o objetivo de melhorar a eficiência catalítica de enzimas, reduzir o tempo e o custo do processo. Nesse sentido, novas metodologias aplicadas para aumentar a atividade da inulinase apresentam alto potencial biotecnológico. O presente estudo investigou o efeito dos TUS, usando uma sonda de ultrassom em três diferentes processos: tratamento enzimático, pré-tratamento do substrato (inulina e sacarose) e sinergismo entre enzima e substrato. Das condições estudadas, o uso de 220 W por 2 minutos promoveu aumento da atividade enzimática em 843%,usando a sacarose como substrato. Quando maiores potências e tempos foram testados, a enzima não melhorou sua atividade, pois isso pode ter afetado sua integridade estrutural. Assim, conclui-se que o uso de tratamentos ultrassônicos em baixas potências e curtos períodos de tempo, apresentam potencial promissor e simples para fins industriais e farmacêuticos.

Palavras-chave: Enzima; Inulina; Substrato; Sacarose; Tratamento combinado

ABSTRACT

The production of fructose and fructooligosaccharides is of extreme industrial importance, since they contemplate the select group of functional foods, being obtained by the catalytic action of inulinases. Ultrasonic treatments (UST) have been widely used in enzymatic reactions in various fields, aiming to improve catalytic efficiency, reduce process time and cost. In this sense, new methodologies applied to increase inulinase activity present high biotechnological potential. The present study investigated the effect of UST using an ultrasound probe in three different processes: enzyme treatment, substrate pre-treatment (inulin and sucrose) and synergism between enzyme and substrate, however on higher values of activity in this substrate. Of the studied conditions, using 220 W during 2 minutes promoted increase of inulinase activity in 843% on sucrose as substrate. When higher powers and times were tested, the enzyme did not improve its activity, as this may have affected their structural integrity.Using ultrasonic treatments in low power and short time condition, our studies provide very useful and simple methods for industrial and pharmaceutical purposes.

Keywords: Enzyme; Inulin; Substrate; Sucrose; Combined treatment

1 INTRODUCTION

The increasing demand for enzymes to industrial applications is connected to the selectivity to the substrate, i.e., their ability to discriminate distinct but structurally similar substrates (Klibanov and Ahen, 1987; Davolli et al., 2004). In this sense, inulinase stands out as an enzyme of high specificity for different substrates, such as sucrose and inulin, and of great relevance for the food and pharmaceutical sector, being applied mainly in the production of syrups with high concentration of fructose, besides others areas such as the production of bioethanol, single-cell, lactic acid, citric acid, Pullulan, tequila, etc. (Singh and Chauhan, 2018). Generally, the substrate binding to the enzyme is a process which occurs with a negative change of Gibbs energy, which contributes to the stabilization of the substrate (Price et al., 2002). In this sense, studies of advanced methods to increase and optimize activity and enzymatic stability have shown great biotechnological potential. The mechanism of action promotes the action of two enzymes, exo and endoinulinase. The first one has the function of separating the fructose terminal units from inulin, which is used for the production of high fructose syrup, while the latter decomposes inulin into inulooligosaccharides (IOS) of varying lengths (Kango and Jain, 2011).

Recently, the application of ultrasound treatment (UST) in biological processes has attracted the attention of the academic community (Bashari et al., 2016; Delgado-Povedano and Luque De Castro, 2015; Martins et al., 2015). For many years it has been used ultrasound as an efficient method for performing enzymatic inactivation (Wang et al., 2018). However, some studies are currently redirecting the perspectives of use, gaining enormous importance in the biotechnology sector for enzymatic enhancement leading to more rapid enzymatic reactions that reveal a positive effect on the enzymatic catalysis (Nguyen and Le, 2013; Wang et al., 2018).

The influence of UST waves on the activity and stability of enzymes has been shown to be specific for each enzyme and dependent on the parameters of sonication (Barton et al., 1996; Sakakibara, 1996; Özbek and Ülgen, 2000). These parameters are related to the cavitation process (a phenomenon best known by the US) and its effect can be observed both in isolation and in a combined form between enzyme and substrate (Delgado-Povedano and Luque De Castro, 2015). One of the effects is purely thermal, the cavitation promotes increase of the temperature of the microzones. Another effect is characterized by the ultrasonolysis of water and other polar liquids forming free radicals. The third effect arises from the movement of microbubbles that collapse and create microstreaming and shock

waves that propagate in the medium promoting mechanical and shear forces allowing molecular alterations (Delgado-Povedano and Luque De Castro, 2015). Ultrasound is considered a method of physical modification but also provides free radicals that act on the enzymes, altering its conformation and functionality and promoting a better interaction between enzyme-substrate (Wang et al., 2018).

According Huang et al. (2017) the application of ultrasound can also alter the characteristics of the substrate, and also in reactions that contain enzyme and substrate combined. The effects produced by the US prior to the enzymatic step causes a reduction in the particle size of the substrate which consequently generates an increase of the catalytic surface reducing the mass transfer limitations (Delgado-Povedano and Luque De Castro, 2015).

In this context, the present study evaluated the positive influence of inulinase activity after UST in sucrose, using experimental design. It was also evaluated the enzymatic activity against different substrates, sucrose and inulin, and investigated the effect of the pretreatment of the UST on the substrates in the absence and synergism of the inulinase activated by the UST.

2 MATERIAL AND METHODS

2.1 ENZYME SOURCE

Commercial inulinase, obtained from *Aspergillusniger* (Fructozyme, mixture of exo-inulinase (EC 3.2.1.80 and endo-inulinase (EC 3.2.1.7)), was purchased from Sigma-Aldrich (USA).

2.2 DETERMINATION OF INULINASE ENZYMATIC ACTIVITY

The determination of inulinase enzymatic activity was carried out using the method described by Miller (1959) with dinitrosalicylic acid (DNS), where it is estimated the release of total reducing sugars present in the assays.

Therefore, 0.5 mL of the sample was added in 0.5 mL of DNS and maintained at 100 °C for 10 minutes and after ice-bath. Subsequently, 8 mL of sodium and potassium tartrate were added to the medium for staining fixation. The samples were quantified in a spectrophotometer at 540 nm.

Brazilian Journal of Development

2.3 EFFECT OF ULTRASOUND ON THE INULINASE ACTIVITY AND THEIR SPECIFICITY

Study of enzymatic pretreatment were performed using Ultrasonic Tip Sonicatorwith maximum power 550 W (Ultronique®)was tests on inulin and/or sucrose as substrate, and subsequent with the acquisition of the best results these were used to achieve an experimental design matrix, with 9 trials and 3 central points, evaluating: exposure time (2 - 10 minutes), power of equipment (ranged from 20 until 80 % or from 220 W until 440 W) and pulses emitted (1 - 3 pulses/minute) in 100 mL of reaction.The levels investigated were determined based on the study by Wang et al. (2012).

In order to evaluate the effect of the ultrasonic waves could alter the specificity of the enzyme, assays with different substrates have been carried out with inulin and sucrose. In this sense, two sampling systems were evaluated: (i) evaluation of the substrate exposure to the ultrasound, in order to understand if the ultrasonic waves could cause alterations in the structure of the inulin and sucrose, facilitating or hindering the subsequent enzymatic action; (ii) the enzyme combined with the substrate on the US treatment.

The experimental conditions for the tests were 2 minutes and 3 pulses, varying the power of the equipment in 40, 60 and 80%. The samples were carried out in triplicate,the results were evaluated in relation to enzymatic activity the relative for each substrate.

The reaction medium was composed of 0.5 mL of the inulinase enzyme solution and 4.8 mL of sodium acetate buffer 2% sucrose (or inulin) 0.1 M pH 4.8. The reaction medium was exposed at 50°C for 2 minutes, after the enzymatic activity was determined.

2.4 DATA TREATMENT

The effect the variables and obtained data were analyzed in STATISTICA 8.0 Software.

3 RESULTS AND DISCUSSION

3.1 ENZYMATIC TREATMENT

The preliminary tests aimed at analyzing only the effects of the ultrasonic waves on the activity of the inulinase enzyme in different substrates, keeping the time constant (2 minutes) and varying only the power efforts of the equipment, (since this is well documented in the literature is a direct cause of improvement or inactivation).

The maximum increase of enzymatic activity on inulin was 205.96% (142.34 U/mL) and on sucrose in 843.49% (213.57 U/mL) in relation to non-treated enzyme. Using intensity of 550 W/cm^2 the enzyme did not improve de activity, may be have altered the enzyme characteristics, affecting it´s structural integrity and decreasing the interaction with the substrate (Figure 1).

In our studies, it is verified that the positive influence of the UST on inulin, modifying the structure that enzyme, generating a greater hydrolysis capacity of the sucrose. The chemical methods revolve around sucrose with hydrolysis in invertase often lead to the decomposition and browning of syrups that use the breakdown products (fructose and glucose) (Magadum and Yadav, 2018). Therefore, an alternative enzyme to improve hydrolysis performance and quality of the final products in fast reaction rates is important for the biotechnological process. In this sense, the present study verified that inulinase combined with the ultrasound technology increases the potential of the hydrolysis of sucrose, showing potential use in food and pharmaceutical industry.

Table 1 shows the variables used (coded and real values) on the relative activity values obtained. A control was carried out with the enzyme without the treatment, obtaining activity of 25.31 U / mL on the sucrose hydrolysis. It is thus verified that the best activity values were found in test 1 and in the center point repetitions. The analysis of the effects allows verifying among the variables monitored in the process, which has influenced the increase or decrease of the response. With the adoption of a 90% confidence level, it was possible to notice that the time of exposure in the system has significant negative effects (t = -2.60) for the best activity values found, the longer the time under influence of the ultrasonic waves the smaller the increase of inulinase activity against sucrose. In this way, short periods of exposure are determinant variables in the conduction of the process, because although the effects of cavitation are important, the contact of the enzyme with them for short periods is enough to accelerate the reaction rates.

TABLE 1

According Zhu (2015), the impact of the effects is directly related to the intensity and duration of the US. The low intensity US causes the enzyme's molecular aggregates to disintegrate into smaller fragments, making active sites more susceptible to contact with the substrate, contributing to the increase in enzymatic activity (Rokhina, Lens and Virkutyte, 2009; Szabó and Csiszár, 2013; Wang et al., 2015). However the high temperature combined

with the high intensity exposes the enzyme in extreme conditions causing its inactivation (Mawson, Gamage and Terefe, 2011; Aghajanzadeh et al., 2016).

3.2 SUBSTRATE AND ENZYME-SUBTRATE COMBINED ULTRASONIC TREATMENT

The effect of UST (220, 330 and 440 W / cm2) on the substrate and a combination of enzymes and substrate was evaluated considering inulinase and sucrose or inulin, respectively. In all cases the best results were obtained when the UST was performed at 330 W/cm2.

Inulinase activity was increased up to 346.94% (239.77 U/mL) relative to the untreated substrate which the inulinase showed 69.11 U/mL of initial activities. When inulin and inulinase were co-treated 341.9% (236.28 U/ mL) of activity was obtained, not showing synergism effect (Figure 2-A). However, a synergistic effect was observed when sucrose and inulinase were co-treated in UST, obtaining an enzymatic activity of up to 1,235.18% (312.62 U/ mL), compared with 869.46% (220.06 U/ mL) when only substrate was treated in UST, and 25.31 U/mL of untreated enzyme activity (Figure 2-B). FIGURE 2

The experiments were based on the substrate pretreatment and the enzyme-substrate combination, aiming to potential the reactions of enzymatic catalysis. In these cases the use of ultrasonic probe functioned as inducer of enzymatic activation, which demonstrates the innovative character of this study, since ultrasonic waves are generally used for enzymatic inactivation, not to mention that it can be considered an eco-friendly method, since it consumes low energy and does not produce any residue, which contributes to its possible implantation in industries (Tao and Sun, 2015; Wang et al., 2016; Wang et al., 2018).

Tests combining the sucrose substrate and enzyme appear to have activated enzymatic catalysis which was not observed when both substrates (inulin and sucrose) were exposed to the ultrasonic probe. Huang et al. (2017) believe that the effects are produced by the cavitation energy but the mechanism by which this occurs is unclear. Their hypothesis is that the increased movement of the liquid molecules causes the access of the substrate to the active site is also increased. This phenomenon can be explained by the possible modifications that the ultrasonic waves can cause in these systems. Researches that performed ultrasonic waves on enzymes justify that, their action breaks down extensive grouping molecules, in addition to causing changes in the secondary and tertiary structures

of the molecule (Yu, Zeng and Lu, 2013; Tian et al., 2016). By specifically treating the result obtained with the substrate-enzyme combination pre-treated in an ultrasonic probe there was supposedly a decrease in the hydrolysis time caused by the reduction of the energy barrier and as result, a greater efficiency and promote the interaction between substrate-enzyme (Cheng et al., 2017;Wang et al., 2018).

The results obtained by inulin or inulin-enzyme complex exposure did not reach the relative activity values found in the sucrose substrate assays, which may be related to/ flexibility of the enzyme against the substrate used. In this case, possibly the conditions used in pretreatment resulted in inhibition of activity and loss of enzyme stability (Özbek and Ülgen, 2000; Fazlena, Norsurayaand Nadiah, 2013; Delgado-Povedano and Luque De Castro, 2015; Wang et al., 2018).

Another reason why substrate-enzyme synergism has shown promising results is related to increase in contact area of active site the enzyme because of cavitation action in the polypeptide structure (Figure 3). In this scenario, it seems that ultrasonic pretreatment stimulates the development of micropores on the surface of enzyme therefore, the enzyme-substrate contact area is amplified which promotes enzymatic activation through the enhancement of the enzyme-substrate affinity (Jin et al., 2016; Waghmare and Rathod, 2016; Wang et al., 2018).

Mulinari et al. (2017) carried out studies with the enzyme lipase to evaluate the effect of ultrasonic waves on the oil hydrolysis reaction. The tests were performed in an ultrasonic bath and resulted in a relative activity of approximately 300%, through an intermediary experimental design condition (25 min, 45ºC and 50% of the total power of the ultrasonic bath, 66 W). Similarly to the results presented in our study, the range of intermediate powers presented promising values of relative activity, indicating the ultrasonic treatment has function of activating enzymatic catalysis and decreasing the reaction time, in this sense, the ultrasonic waves can act as an efficient pretreatment aimed at the industrial application, making the process efficient, reducing steps and consequently costs (Wang et al. 2018).

.

4 CONCLUSIONS

Sucrose is a substrate that comes from sources with low costs when compared to inulin. From the tests performed it was verified that the conversion of sucrose to fructose was quite high when submitted to ultrasonic waves in synergism with the enzyme inulinase reaching

relative activity of 843.8% in conditions that use little time and low energy of the ultrasonic probe.

These results allow us to define operation conditions in terms of residual activity that results in higher values aiming at using it as catalysts in reactions of interest, making it possible, based on the presented data and the results reported in the literature, to investigate the potential of ultrasound technology in achieving such reactions.

FINANCIAL SUPPORT

The authors thank CNPq, CAPES, CAPES-PNPD and FAPERGS for the financial support of this work and scholarships.

CONFLICT OF INTEREST

All authors do not have any actual or potential conflict of interest.

REFERENCES

Aghajanzadeh, S., Ziaiifar, A. M., Kashaninejad, M., Maghsoudlou, Y., Esmailzadeh, E. (2016). Thermal inactivation kinetic of pectin methylesterase and cloud stability in sour orange juice. *Journal of Food Engineering*, v.185, p.72-77.

Barton, S., Bullock, C., Weir, D. (1996). The effects of ultrasound on the activities of some glycosidase enzymes of industrial importance. *Enzyme and Microbial Technology*, v.18, p.190-194.

Bashari, M., Jin, Z., Wang, J., Zhan X. (2016). A novel technique to improve the biodegradation efficiency of dextranase enzyme using the synergistic effects of ultrasound combined with microwave shock. *Innovattive Food Science & Emerging Technologies*, v.35, p.125-132.

Cheng, Y., Liu, Y., Wu, J., OforiDonkor, P., Li, T., Ma, H. (2017). Improving the enzymolysis efficiency of potato protein by simultaneous dual-frequency energy-gathered ultrasound pretreatment: thermodynamics and kinetics. *UltrasonicsSonochemistry*, v. 37, p. 351–359.

Davolli, P., Mierau, V., Weber, R. W. S. (2004). Carotenoids and fatty acids in red yeasts Sporobolomycesroseus and *Rhodotorulaglutinis*. *Applied Biochemistry & Microbiology*, v.40, p.392-397.

Decker, H., Schweikardt T., Nillius D., Salzbrunn U., Jaenicke E., Tuczek F., (2007). Similar enzyme activation and catalysis in hemocyanins and tyrosinases, *Gene*, v.398, p.183–191.

Delgado-Povedano, M. M, Luque De Castro, M. D. (2015). A review on enzyme and ultrasound: A controversial but fruitful relationship. *AnalyticaChimica Acta*, v.889, p.1-21.

Fazlena, H., Norsuraya, S., Nadiah, S.N. (2013). Ultrasonic Assisted Enzymatic Reaction: An Overview on Ultrasonic Mechanism and Stability Activity of the Enzyme. *Business Engineering and Industrial Applications Colloquium*, IEEE, Langkawi, Malaysia

Huang, G., Chen, S., Dai, C., Sun, L., Sun, W., Tan, Y., Xiong, F., He, R., Ma, H. (2017). Effects of ultrasound on microbial growth and enzyme activity. *UltrasonicsSonochemistry*, v.37, p.144-149.

Jin, J., Ma, H., Wang, W., Luo, M., Wang, B., Qu, W., He, R., Owusu, J., Li, Y. (2016). Effects and mechanism of ultrasound pretreatment on rapeseed protein enzymolysis. *Journal of the Sicence of Food and Agriculture*, v. 96, p. 1159–1166.

Kango, N., & Jain, S. C. (2011). Production and Properties of Microbial Inulinases: Recent Advances. Food Biotechnology, v.25, p.165–212. doi:10.1080/08905436.2011.590763.

Klibanov, A. M., Ahen, T. J. (1987). Protein engineering: Thermal stability of proteins. In: Oxender DL, Fox CF, Editors. Alan R. Liss, New York, p.213–218.

Magadum, D. B, &Yadav, G. D. (2018). Fermentative production, purification of inulinase from Aspergillusterreus MTCC 6324 and its application for hydrolysis of sucrose. *Biocatalysis and agricultural biotechnology*, v.14, p.7-299.

Martins, M., Azoia, N., Silva, C., Cavaco-Paulo, A. (2015). Stabilization of enzymes in micro-emulsions for ultrasound processes. *Biochemical Engineering Journal*, v.93, p.115-118.

Mawson, M., Gamage, N. S., Terefe, K. (2011). Ultrasound in enzyme activation and inactivation. Feng H., Barbosa-Canovas G., Weiss J. (Eds.), *Ultrasound Technologies for Food and Bioprocessing, Springer*, p.369-404.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, v.31, p.426.

Mulinari, J., Venturin, B., Sbardelotto, M., DallAgnol, A., Scapini, T., Camargo, A.F., Baldissarelli, D.P., Modkovski, T.A., Rossetto, V., Dalla Rosa, C., Reichert Jr, F.W., Golunski, S.M., Vieitez, L., Vargas, G.D.L.P., Dalla Rosa, C., Mossi, A.J., Treichel, H. (2017). Ultrasound-assisted hydrolysis of waste cooking oil catalized by homemade lipases. *UltrasonicsSonochemistry*, v.35, p.313–318.

Nguyen, T. T. T., Le, V. V. M. (2013). Effects of ultrasound on cellulolytic activity of cellulose complex. *International Food Research Journal*, v.20, p.557–563.

Özbek, B., Ülgen, K. O. (2000). The stability of enzymes after sonication. *Process Biochemistry*, v.35, p.1037-1043, 2000.

Price, N. C., Dwek, R. A., Ratcliffe, R. G., Wormald, M. R. (2002). Principles and problems in physical chemistry for biochemists. *Oxford University* Press3 Eds. 424 pages.

Rokhina, E. V., Lens, P., Virkutyte, J. (2009). Low-frequency ultrasound in biotechnology: state of the art. *Trends in Biotechnology*, v.27, p.298-306.

Sakakibara, M., Wang D., Takahashi, R., Takahashi, K., Mori, S. (1996). Influence of ultrasound irradiation on hydrolysis of sucrose catalyzed by invertase. *Enzyme and Microbial Technology*, v.18, p.444-448.

Singh, R. S., Chauhan, K. (2018). Production, purification, characterization and applications of fungal inulinases. *Current Biotechnology*, v.7, p.242-260.

Szabó, O. E., Csiszár, E. (2013). The effect of low-frequency ultrasound on the activity and efficiency of a commercial cellulase enzyme. *Carbohydrate Polymers*, v.98, p.1483-1489.

Tao, Y., Sun, D.W. (2015). Enhancement of food processes by ultrasound: a review, Crit. Rev. *Food Science & Nutrition*, v. 55, p. 570–594.

Tian, M.L., Fang, F., Du, M.Y., Zhang, F.S. (2016). Effects of pulsed electric field (PEF) treatment on enhancing activity and conformation of alpha-amylase. *The Protein Journal*, v. 35, p.154–162.

Waghmare, G.V., Rathod, V.K., (2016). Ultrasound assisted enzyme catalyzed hydrolysis of waste cooking oil under solvent free condition. *UltrasonicsSonochemistry*, v. 32, p. 60–67.

Wang, D., Yan, L., Ma, X., Wang, W., Zou, M., Zhong, J., Ding, T., Ye, X., Liu, D. (2018). Ultrasound promotes enzymatic reaction by acting on different targets: Enzymes, substrates and enzymatic reaction systems. International Journal of Biological Macromolecules, v. 119, p. 453-461.

Wang, W., Ma, X., Jiang, P., Hu, L., Zhi, Z., Chen, J., Ding, T., Ye, X., Liu, D. (2016). Characterization of pectin from grapefruit peel: a comparison of ultrasound-assisted and conventional heating extractions. *Food Hydrocolloids*, v. 61, p. 730–73.

Wang, Z., Lin. X., Li, P., Zhang, J., Wang, S., Ma, H. (2012). Effects of low intensity ultrasound on cellulase pretreatment. *Bioresource Technology*, v.117, p.222-227.

Yu, Z. L., Zeng, W. C., Lu, X. L., (2013). Influence of ultrasound to the activity of tyrosinase, UltrasonicsSonochemistry, v.20, p.805–809.

Zhu, F. (2015). Impact of ultrasound on structure, physicochemical properties, modifications, and applications of starch. *Trends in Food Science and Technology*. v.43, p.1-17.

Brazilian Journal of Development

Table 1. Matrix of the experimental design (real and coded values) performed with the activity of inulinase after ultrasound treatment (Crude enzyme activity. 25.31 U/mL).

Assay	Time (min)	Intensity (W/cm2)	Pulse	RelativeActivity (A/Ao)*100
1	(-1) 2	(-1) 220	(-1) 1	843.8
2	(+1) 10	(-1) 220	(-1) 1	742.8
3	(-1) 2	(+1) 440	(-1) 1	724.5
4	(+1) 10	(+1) 440	(-1) 1	214.4
5	(-1) 2	(-1) 220	(+1) 3	708.8
6	(+1) 10	(-1) 220	(+1) 3	30.6
7	(-1) 2	(+1) 440	(+1) 3	598.4
8	(+1) 10	(+1) 440	(+1) 3	101.5
9	(0) 6	(0) 330	(0) 2	722.6
10	(0) 6	(0) 330	(0) 2	819.2
11	(0) 6	(0) 330	(0) 2	819.2

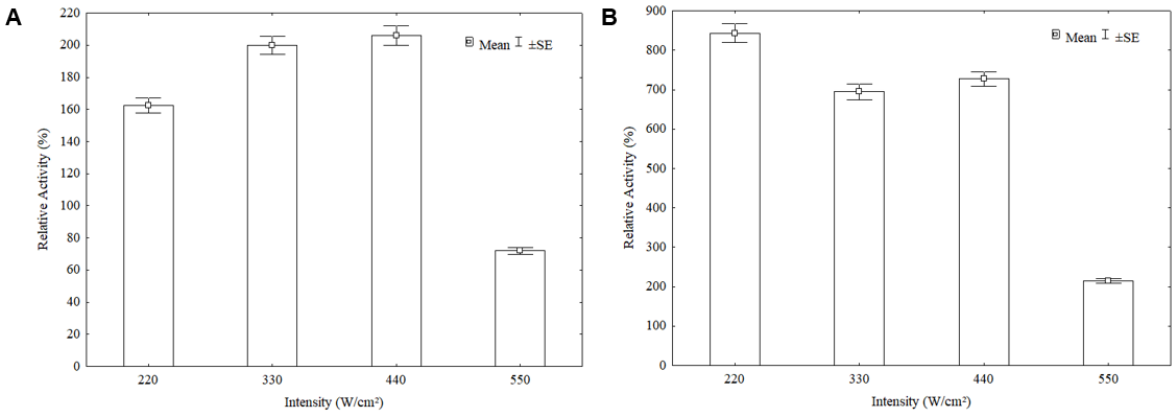

Figure 1: Inulinase enzyme exposure in ultrasound system and subsequent application on Inulina (A) and Sucrose (B).

Brazilian Journal of Development

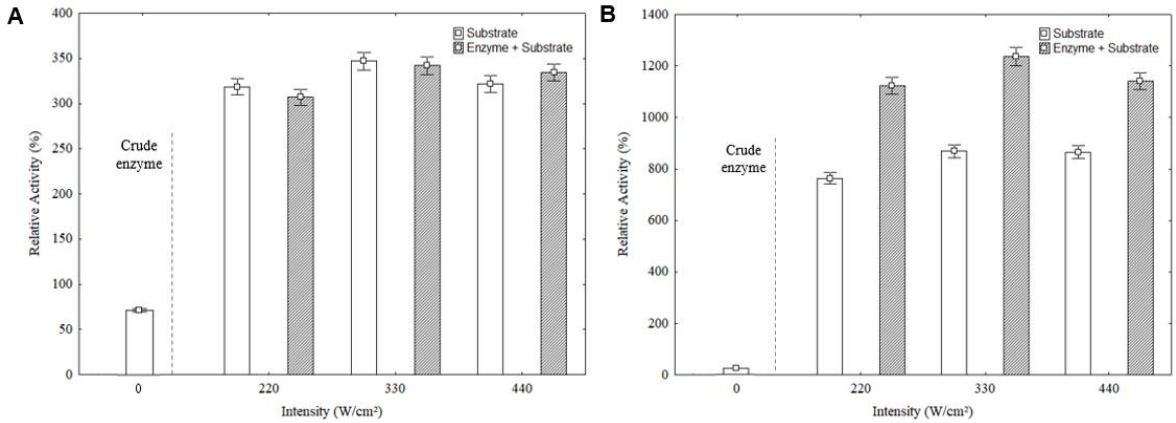

Figure 2. Relative activity of inulinase enzyme, considering substrate treatment and synergism between enzyme (inulinase) and substrate inulin (A) and sucrose (B) exposure in ultrasound system cooperated with crude inulinase activity.

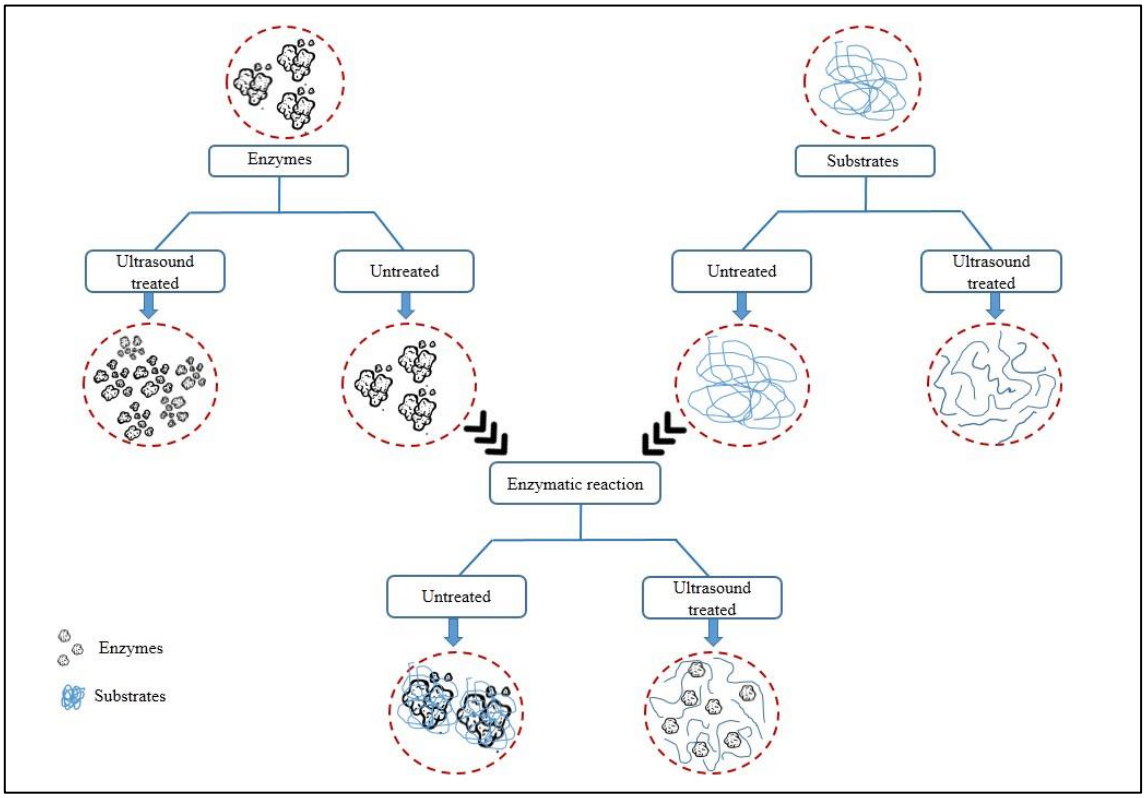

Figure 3. Schematization of the possible effects produced by the ultrasound on theenzyme, on the substrates and on the combination of both.